A STUDY OF CHANGES IN FLUORESCENCE AND PROTEIN SOLUBILITY IN GERM-DAMAGED WHEAT

by

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INTRODUCTION

A type of damage occurring in wheat stored at high levels of moisture and temperature has been known in the trade as "sick" wheat. The affected kernels take on a dull appearance, and the germs exhibit various degrees of discoloration from light brown to black. The germs of such kernels are non-viable and are usually invaded by certain molds, principally various species of Aspergillus (Geddes, et al, 11). The fat acidity usually increases with "sick" wheat deterioration. A musty odor sometimes accompanies this type of damage.

At present Federal and licensed grain inspectors estimate the percentage of damage by visual determination of the number of discolored germs found in approximately ten grams of grain. (Cole, 9).

This technique is highly empirical since the evaluation by visual inspection will vary from one individual to another and because it fails to detect the early stages of deterioration that precede the appearance of sick wheat.

The work of Cole and Milner (10) indicates that the formation of sick wheat is associated with the production in germ of wheat of substances which are strongly fluorescent, apparently produced by a Maillard or browning reaction.

Cole suggested accordingly that a quantitative method for determining sick wheat might be developed based on the increase in fluorescence of aqueous extracts of damaged grain. Although he found a fairly good correlation between fluorescence value and the sick kernel content of commercial wheat samples as evaluated by Federal and licensed grain inspectors, the test does not appear sufficiently sensitive for practical use.

The reason for this appears to be that the deterioration in its critical initial stages is essentially confined to the germ which constitutes only from 2 to 5 percent of the entire kernel. Thus the appearance of a small amount of a fluorescing substance in this minor quantity of tissue would be difficult to detect because it would be strongly overshadowed by the natural fluorescence characteristic of the balance of the kernel. This natural fluorescence, apparently the composite of several fluorescing compounds in wheat, varies appreciably among wheat samples of different origin. The data of Cole (9) indicate differences of up to 10 scale units fluorescence among the extracts of apparently sound wheat samples. Small changes could not be detected because one would not know whether to attribute the observable fluorescence increase to the browning products or a higher concentration of one of the naturally occurring substances.

If the latter fluorescence could be masked, quenched, or the responsible naturally occurring substances removed, the fluorescence due to the browning products might be accurately measured when present only to a slight degree.

An alternative approach to the detection of sick wheat damage might be in the investigation of changes in other chemical constituents of the embryo, which undergo modifications prior to the development of fluorescent compounds.

REVIEW OF THE LITERATURE

The "sick" wheat condition, as distinct from other types of deterioration, had been recognized since 1921.

Thomas (Cole, 9) observed a decrease of wheat seed viability in the presence of certain species of fungi, which he believed was related to the "sick" condition. Thomas made germination tests on wheat after treating the seed with filtrates of pure cultures of 13 species and strains of molds which

are commonly found in grain. While all the filtrates lowered the germination somewhat, Aspergillus flavus had a very pronounced toxic effect. He apparently paid no attention to the characteristic discoloration of the germ in sick wheat and concluded that sick wheat was grain which had lost its viability due to toxic products elaborated by molds.

Milner et al (23) found differences in the kind of microflora and their percentage distribution on samples of commercial sick wheat compared to sound wheat. Sick or germ-damaged wheat samples showed very low germination values and were found to be infected principally with Aspergillus glaucus, Penicillium and Aspergillus candidus. In contrast, sound wheat samples from the same lots of grain were largely contaminated with Alternaria which disappeared when stored under moisture conditions favorable to the proliferation of the Aspergilli. Sick kernels were produced by storing wheat containing 18 percent moisture under atmospheres of carbon dioxide, nitrogen and oxygen, in sealed containers. Only under oxygen did molds proliferate, whereas sick kernels developed under all three atmospheres.

The deteriorative effect of mold growth is manifested principally in lipolytic activity and appears to be additive to other deteriorative processes responsible for "sick" wheat.

Swanson, (30) in studying the role of moisture, time and temperature in stored wheat had found, similarly, that inhibiting mold growth did not prevent sick wheat deterioration as measured by the baking test. Among the chemical changes observed in sick wheat were increases in fat acidity, which can be measured by the amount of standard alkali necessary for neutralization of fat extracts.

The development of fat acidity or rancidity is however, related to air supply. In the entire exclusion of air this will not develop. Hence the conditions which favor the development of mold growth and fat acidity are closely related, but absence of high acidity is not proof of lack of damage. (30, 31).

Carter and Young (7) also produced sick wheat in the absence of molds. Wheat containing 12.2 percent moisture stored at 40°C developed sick wheat symptoms when stored 279 days or longer, but not when stored at a lower temperature. A small percentage of sick wheat was produced in 32 days in wheat containing 18.6 percent moisture when stored at 5°C, and up to 100 percent sick kernels when stored at higher temperatures and for longer periods of time (8).

Cole and Milner (10) have shown in recent work that the light absorption spectrum of extracts of normal wheat was characterized by a peak at 270 mu and an inflection at 325 mu, both of which increased slightly when the grain became germ-damaged. The damaged wheat extract also showed a marked increase in fluorescence over that of the sound wheat extract.

The dark pigment in the germ of the sick wheat and in the browned germ of laboratory storage appears to be responsible for the increase in light absorption and fluorescence. It is suggested that this pigment is the product of a sugar-amino and/or protein condensation, better known as the Maillard or browning phenomenon.

Another general characteristic of Maillard condensation products, which Cole observed in his extracts of browned germ, was that the brown pigments could be strongly absorbed by such materials as magnesium oxide, Supercel, activated alumina, and Florisil (Fuller's earth preparation).

The work of McDonald (22) lends considerable additional support to the theory of a browning reaction between reducing sugars and nitrogenous compounds as postulated by Cole and Milner (10). In storage experiments designed to determine which of the three major constituents of wheat germ, sugar (principally sucrose and raffinose), protein, and oil, might be involved in the darkening of germ, McDonald found that extraction of water soluble substances, primarily sugars, greatly inhibited the development of browning upon prolonged storage at high humidities. Ether extraction of the oils did not prevent the usual development of darkening at such high humidities. This indicated that sugars are involved in the characteristic browning reaction whereas the oils are not. This worker observed, furthermore, that the wheat germ proteins became decreasingly peptizable in potassium sulfate solution as browning increased. McDonald suggested that in the course of the browning reaction the protein may have been denatured, hydrolyzed, or combined with other compounds to form non-peptizable nitrogen containing compounds.

The chemistry of browning reactions has been thoroughly reviewed by Hodge (13), Hodge and Rist (14), and by McDonald (22) in relation to the sick wheat condition.

Three broad types of browning reactions are recognized in food technology. The most common type, carbonyl-amino reactions, includes the reactions of aldehydes, ketones, and reducing sugars with amines, amino acids, peptides and proteins.

Another type, called caramelization, occurs when polyhydroxycarbonyl compounds (sugars, polyhydroxycarboxylic acids) are heated to relatively high temperature in the absence of amino compounds. This type of browning characteristically requires more energy to get started than the carbonyl-

amino reactions, other conditions being equal.

Neither carbonyl-amino nor caramelization reactions are dependent upon the presence of oxygen to produce browning.

A third type of browning frequently encountered by the food processor is the group of oxidative reactions, which, for instance, convert ascorbic acid and polyphenols into di-or polycarbonyl compounds. These oxidations may or may not be enzyme-catalyzed. (14)

Increased attention has been given the Maillard reaction in recent years since it has been established without doubt that such changes are often connected with considerable lowering of the nutrient effect.

Thufel and Iwainsky (31), in a critical study of the Maillard reaction in model systems, observed that the individual participants in the reaction underwent relatively slight changes in concentration after prolonged heating. During heat treatment of mixed model systems containing glucose and amino acids, a strong decline in the concentration of both reactants, accompanied by rapid coloring, was measured.

This type of change, resulting in an evident decrease in the nutrient effect, has been found to occur in various stored foods and food products, such as dried milk, dried egg, dried meat, dried and canned fruit (Hewston, et al, 12) and different grain products. It is generally related to adverse conditions of temperature, moisture and length of storage. Feeding of heated foods to experimental animals showed amino acid deficiencies. The foreign brown materials, though non-toxic, were eliminated and not utilized by the body. (31)

An example of this type of change and its relation to time and temperature

of storage in a natural system was the work of Hodson on canned, evaporated milk, stored for varying lengths of time and at different temperature levels. At 4°C there were no losses of amino acids following two years of storage. At 38°C there were measurable losses of tryptophan, lysine, histidine and arginine after one year of storage. After two years, the losses were large, being tryptophan 12 percent, lysine 29 percent, histidine 29 percent and arginine 28 percent. There were also important deteriorative changes in appearance and palatibility after long storage at 38°C (Hodson, 15).

Exposure to heat and moisture deepened the color of soybean meal in a regular manner (Beckel, et al, 5). This change occurred simultaneously with the denaturation of protein which could be measured by a progressive insolubility in water and dilute salt solutions. The authors, Beckel, Bull and Hopper, made the interesting observation that exposure to heat and moisture deepened the color of the meal in a regular manner, and that this change occurred simultaneously with the denaturation of the protein (5). Although this darkening may not be the direct result of protein denaturation, it would be due to interaction between protein and carbohydrate.

The data of Beckel et al indicate that a critical temperature probably exists at each relative humidity below which very little denaturation takes place, but above which insolubilization is rapid (Markley, 21).

It has been shown that when wheat and its milling products are stored, marked changes occur in the properties of the proteins (17, 18). The changes include (1) decrease in the solubility of the proteins in various dispersing agents, such as neutral salt solutions and alcohol; (2) proteolysis, or breaking down of the native proteins into entities of smaller molecular dimensions; and (3) decrease in digestibility when treated with pepsin and

trypsin in vitro. The extent of the changes depend on temperature, type of container, and on whether the whole kernels or their milling products are stored (Jones and Gersdorff, 16). Temperature was a most important factor. The decrease in solubility at 76°F was more than double that at 30°F in the same time interval. Jones, et al (17-18) noted that protein denaturation was most rapid during the early periods of storage. The changes that occurred during the first month of storage were in some instances as much as three-fourths of those found at the end of 24 months. There were no signs of mold invasion or any other type of damage in the grain.

THE PROBLEM

The over-all purpose of the present research was to find an analytical method capable of accurately detecting the degree of damage in a sample of "sick" wheat; particularly in its critical initial stages.

A first approach was to investigate the use of adsorbents to separate the products of deterioration associated with the browning of wheat germ from naturally fluorescing substances in wheat and thus increase the sensitivity of the fluorometric technique developed by Cole (9).

Another alternative was the detection of some physical, structural or chemical change which necessarily precedes the formation of the browning product, the degree of which might constitute a measure of "sick" wheat deterioration. Protein denaturation, as evidenced by progressive protein insolubilization, was investigated as a sensitive index of incipient deterioration.

MATERIALS

Unbleached flour from sound wheat was obtained from the experimental mill at Kansas State College. Unprocessed granular wheat germ was supplied by General Mills Inc., Minneapolis, Minnesota. A few samples of commercial wheat having varying degrees of germ damage, were acquired from the Federal Grain Inspection Office, Kansas City, Missouri.

For the study of changes in peptizable protein with temperature, time and moisture contact during storage, six samples of sound wheat of the 1954 Kansas crop were selected.

These sound wheats were characterized as follows:

Serial No.	Variety	Source	Test Weight (lb.)	Moisture (%)
C-1	Ponca	Manhattan	61.6	8.20
C-2	Concho	Manhattan	62.6	8.26
C-5	Ponca	Hutchinson	61.0	8.21
C-6	Concho	Hutchinson	63.0	8.61
C-9	Ponca	Fort Hays	58.7	8.47
C-10	Concho	Fort Hays	58.3	8.25

The following adsorbents were investigated:

Florisil, 200 mesh, a Fuller's earth preparation, obtained through courtesy of the Floridin Company, Tallahassee, Florida.

Lloyd Reagent, a form of hydrated aluminum silicate, Fisher Scientific Company.

Isco Adsorbol N-100

Isco Adsorbol A-420

Supercell, John's Mannsville Product

Norite, and about 12 others, which were only tried once, and having been found ineffective under this particular set of conditions, were not employed again.

The intermediate Wiley mill with a No. 30 screen was utilized for grinding the samples.

Fluorescence measurements were made with the Coleman Electron Photofluorometer with Vitamin B_1-S and PC-1 filters transmitting at 345 mu. Sodium Fluorescein (0.1 p.p.m.) was used to standardize this instrument, the dial being set at 50 with this solution.

Percent transmittancy measurements were carried out with the Coleman Universal Spectrophotometer at a wave length of 530 mu.

The Macbeth pH meter was utilized for pH measurements.

METHODS

Moisture Determinations

When the moisture content was less than 14 percent, the one-hour air oven method, as outlined in Association of Official Agricultural Chemists (A.O.A.C.), page 192, was followed. (3) For samples which had been conditioned to moisture levels above 14 percent, a standard two-stage method, described in Cereal Lab. Methods, page 6, was adopted. (2)

Preparation of Germ Samples Representing Progressive Browning

Wheat germ samples representing progressive stages of browning were prepared by heating moistened germ in the oven at 50°C for various time intervals. Conditioning of germ to various moisture levels did not involve the simple addition of water to germ since the material would lump. In order to obtain a uniform distribution of moisture the water must be applied as a

fine spray while the germ is in motion. The procedure of McDonald was used as follows: (22).

200 g of sound germ (9.6 percent moisture) were brought to a 20 percent moisture content by slowly dropping 30 cc distilled water from a burette into a can containing the material, while it was being rapidly stirred by means of an electrical stirring device. Stirring was continued until all lumps had been broken up. Four fractions representing increasingly advanced stages of browning were prepared: The first fraction of approximately 50 g not subjected to oven heating, was placed into a sealed container and stored in the refrigerator. The rest of the wetted germ was placed in a sealed Erlenmeyer flask and processed in an air oven at 50°C. Portions of approximately 50 g were withdrawn after 8, 24 and 48 hour processing intervals and stored in the same manner as the first fraction. Thus a series of four samples representing progressive stages of browning was obtained under controlled conditions of time, temperature and moisture, with the first fraction serving as a control or blank, since it had not been subjected to heating.

Moisture, fluorescence, adsorption and turbidity determinations were made on each of these four fractions. The moisture had to be determined following storage at oven temperatures to verify if there had been changes in the moisture content of any one or all of the fractions after tempering. Since browning is also related to moisture content a variation of only 1 to 2 percent would already significantly affect the rate at which browning developed and in the preparation of this series the moisture factor had to be kept constant in the four fractions in order to be sure that the extent of any change was a function solely of heating time at a constant temperature, compared to the first fraction or control.

Method of Preparing Intact Wheat Kernels Representing Progressive Degrees of "Sick" Wheat Deterioration

Wheat samples were conditioned to moisture levels of 14, 16, 20 and 22 percent by adding the calculated amount of distilled water to the weighed grain, mixing it thoroughly at hourly intervals and letting it stand overnight. Samples representing progressive stages of browning were obtained by storing the tempered wheat samples in their sealed containers at different temperatures and for various time intervals. Following storage in the oven at elevated moisture contents the samples were dried to a uniform moisture content and ground to pass through a No. 30 sieve for all subsequent determinations.

Several storage experiments with intact kernels were conducted. Five series representing increasingly advanced stages of browning as a function of heating time were obtained using five samples of sound wheats of different origins, tempered to a moisture content of 22 percent. A portion of each sample was withdrawn before storage at oven temperatures and the rest of the five samples were stored in the air oven at 65°C. Subsequent portions were withdrawn at 24 hour intervals, the last one after 120 hours. In another storage experiment designed to indicate the sensitivity of the turbidity method for detecting early changes during storage, as compared to the fluorometric procedure, one wheat variety, conditioned to a moisture content of 22 percent was stored in the oven at 65°. Samples were withdrawn after the first hour and two-hour intervals up to 11 hours. In a third storage experiment, one variety (8.26 percent moisture) was tempered to moisture levels of 14, 16, 18, 20 and 22 percent. At each of these moisture levels the early effect of storage at temperatures of 100°C, 65°C, 50°C and 30°C was observed and the

time interval was recorded when changes at each temperature and moisture first became evident.

Measurement of Fluorescence

For the analysis of fluorescence essentially the procedure of Cole was followed: The sample size varied from 5.000 g for flour samples to 2.000 g for ground samples of whole wheat, degerminated wheat, sectioned germ-containing ends and granular wheat germ. The samples were extracted for 45 minutes with 15 ml. 0.2 N HCl. The mixture was separated in 15 ml. centrifuge tubes by centrifuging at 1500 rpm for five minutes. The extracts were filtered and clarified by shaking vigorously (60-80 times) with 5 ml. chloroform. A second fifteen minute centrifugation ensued. The desired number of milliliters of the clear upper layer was pipetted off, transferred to a volumetric flask and diluted to volume.

Measurement of Adsorption

For adsorption studies, the clarified and diluted extract from fluorescence determinations was divided into aliquots. A weighed amount of adsorbent (1 g./ 25 ml. for flour and 4 g./ 50 ml. for wheat meal and granular wheat germ) was added to each aliquot and mixed well. The adsorbent was allowed to act for 1½ hours and was then filtered off. The adsorption was evaluated as the difference in fluorescence readings between the aliquot not subjected to treatment with adsorbent and that of the filtrate after the adsorbent had been allowed to act.

Turbidity Method

A turbidity method was developed in the course of this work to follow the changes in peptizable protein concurrent with storage at varying moistures and temperatures. The detailed procedure will be described under Experimental.

EXPERIMENTAL

The experimental studies, aimed at developing a sensitive measurement of "sick" wheat deterioration, were divided into two areas of work: (I) A study of the use of adsorbents to separate the fluorescing products developed as a result of a Maillard reaction from naturally fluorescing pigments in wheat; (II) Development of a turbidity method as a criterion of protein solubility changes apparently related to "sick" wheat deterioration - and its correlation with the characteristic increase in fluorescence found in progressive browning of wheat.

Use of Adsorbents to Separate the Products of Deterioration Associated with "Sick" Wheat

Effect of Adsorbents on Flour at Two Concentrations. With the idea of finding an adsorbent which would selectively remove the naturally fluorescing substances in wheat, a qualitative study was carried out of the effect of a number of different adsorbents on unbleached flour. The latter material was chosen because it would be representative of the fluorescing materials in the endosperm of sound wheat.

For the first trial, one ml. of the 15 ml. - 5 g. extract was diluted to 100 ml. for fluorescence measurements. At this dilution the amount of fluorescing material was too slight to study the possible decrease in the fluorescence due

to the effect of adsorbents. The dilution was therefore decreased to the ratios of 1 ml. to 25 ml. and 2 ml. to 25 ml. The results obtained with several adsorbents are shown in Table 1.

Table 1. Effect of Adsorbents on the Fluorescence of Flour Extracts

Treatment	Fluorescence (scale units)			
	Dilution 1:25	Change	Dilution 2:25	Change
None	10	-	21	-
Florisil	7	3	7	14
Supercell	10	0	13	8
Isco Adsorbol N-100	2	8	3	18
Isco Adsorbol A-420	5	5	8	13
Lloyd Reagent	0	10	0	21
Norite	0	10	0	21

Table 1 indicates that a one ml. portion of a 15 ml. 0.2 N HCl extract of a 5 g. sample of unbleached flour, diluted to 25 ml. with 0.2 N HCl, would give a fluorescence of 10 scale units. A 2 ml. extract, at the same dilution, would give a reading of 2 1; or approximately 10 scale units increase for each milliliter of flour extract. Therefore, if following treatment with an adsorbent the fluorescence reading diminished to zero, it would indicate complete removal of the fluorescence in flour extracts by the adsorbent. Accordingly, Lloyd Reagent and Norite completely removed the fluorescent materials in unbleached flour. Isco Adsorbol N-100 showed strong adsorption. Isco Adsorbol A-420 yielded definite adsorption. Florisil and Supercell

indicated partial adsorption on the 2 ml. flour extracts. The effect of the two latter adsorbents on the 1 ml. extracts was insignificant.

It is inferred, from Table 1, that adsorption will increase with concentration of the extracts. An adsorbent with no effect on a small amount of fluorescing material, may remove part of it when the same material is present at higher concentrations. The next step was to investigate the action of these adsorbents on a "sick" wheat extract and on a mixed "sick" wheat plus flour, extract.

Differential Adsorption of Fluorescence Due to Flour and "Sick" Wheat. It seemed possible to determine the utility of the adsorbents previously studied to differentiate between the fluorescence of "sick" wheat and that of endosperm of undamaged wheat, for the following reason:

Results up to this point indicated that a one ml. portion of a 15 ml. 0.2 N HCl extract of a 5 g. sample of unbleached flour, diluted to 25 ml. with 0.2 N HCl, would give a fluorescence reading of 10. It was assumed that of the total fluorescence reading of a strongly fluorescing "sick" wheat extract, similarly obtained and diluted, 10 scale units would likewise be due to fluorescing substances other than the "sick" wheat products. Thus if treatment with Lloyd Reagent, Norite and/or Isco Adsorbol reduced the fluorescence significantly more than 10 for the "sick" wheat extract and more than 21 (Table 1) for the mixture of flour plus "sick" wheat extract, it would mean that the fluorescing materials associated with "sick" wheat had been partially or totally removed.

Florisil and Supercell appeared particularly interesting in this regard because of their insignificant effect on the fluorescence of the endosperm. Any fluorescence-diminishing effect by these adsorbents on an equivalent sample containing "sick" wheat extract, might therefore be considered due

to selective adsorption of the fluorescent products of "sick" wheat.

Following the conditions of equivalence as specified above, fluorescence measurements indicating the effect of adsorbents were made on:

(a) Mixture of "sick" wheat extract plus flour extract;

(b) The "sick" wheat extract alone;

(c) The flour extract measurements of Table 1, inserted for comparison.

Results are recorded in Table 2.

From Table 2 it appears that approximately 50 scale units of fluorescence-a value arrived at by subtracting the fluorescence reading of flour from that of "sick" wheat, or by subtracting from the mixture of extracts the value of 2 0, corresponding to a 2 ml. flour extract - could be considered as due to the browning products of "sick" wheat. With this criterion, to fulfill conditions of complete and selective removal of the fluorescence due to "sick" wheat products, an adsorbent would have to (1) Give the same adsorption value, approximately 50, for both the mixture of "sick" wheat plus flour extracts and the "sick" wheat extract alone; (2) Give an adsorption approaching zero (0) for the flour extract alone. To fulfill conditions of complete and selective removal of the natural fluorescence stemming mainly from the endosperm of undamaged wheat, an adsorbent would have to give an adsorption of 10 for the flour extract and approximately 20 and 10 for the mixture of flour plus "sick" wheat extracts, and "sick" wheat alone, respectively.

Table 2. Effect of Adsorbents on Mixtures of Extracts of Unbleached Flour and "Sick" Wheat, on Extracts of "Sick" Wheat alone and on Extracts of Flour Alone.

	Fluorescence (Scale units)					
	Mixture of "sick" wheat and unbleached flour		"Sick" wheat		Unbleached flour	
Treatment	Reading	Change	Reading	Change	Reading	Change
None	70		60		10	
Florisil	18	-52	17	-43	7	-3
Isco Adsorbol N-100	22	-48	12	-48	2	-8
Lloyd Reagent	9	-61	4	-56	0	-10
Norite	0	-70	0	-60	0	-10
Supercell	63	- 7	51	- 9	10	0

None of the adsorbents studied removed the fluorescence of flour without also removing that associated with "sick" wheat. Norite indiscriminately adsorbed both pigments. Supercell, following a series of tests, was found to adsorb slightly the naturally occurring fluorescing materials in wheat. Isco Adsorbol N-100 gave the same adsorption value of 48 for both the "sick" wheat alone and the mixture of extracts. However, it had also yielded strong adsorption when used with flour alone. Lloyd Reagent showed similar effects. Florisil appeared most promising in providing an insignificant adsorption value for the flour extract and high values for the mixture of "sick" wheat plus flour and "sick" wheat alone, extracts. However, since adsorption was greater for the mixed "sick" wheat and flour extracts than for the "sick" wheat extract alone, whereas they should have been the same, the possibility that fluorescing materials of the endosperm had also been adsorbed could not be ruled out.

Sources of Error in Measuring the Adsorption. Since the value for adsorption was arrived at by taking two fluorescence readings, it is obvious that any foreign matter or change affecting the fluorescence reading, would result in an erroneous estimation of the adsorption. For instance, if following treatment with an adsorbent the pH of the filtrate had decreased, this would very likely result in a lowered fluorescence reading, giving a high adsorption value. The contrary would occur if the pH had increased. Another source of error would be the contribution of fluorescence by the adsorbent. Both of these possibilities were investigated.

A check on possible pH changes after addition and filtration of the adsorbents revealed that for Lloyd Reagent and Isco Adsorbol N-100 these changes remained within 0.1 of a pH unit; for Florisil and Norite, within 0.2 and 0.3 of a pH unit, respectively. It was observed that, everything else remaining equal, a 0.1 to 0.2 change in pH may change the fluorescence reading up to 2 scale units.

A blank determination was run on those adsorbents which appeared promising, to investigate whether the fluorescence reading of the extraction medium was changed. Data obtained are recorded on Table 3.

Table 3. Effect of Adsorbents on Fluorescence of Extraction Medium: 0.2 N HCl Solution.

Adsorbent	Fluorescence (scale units)
None	10
Florisil	14
Isco Adsorbol N-100	10
Lloyd Reagent	10
Norite	10

This indicated that Florisil contributed some fluorescing materials to the solvent.

Studies up to this point revealed that, in general, the fluorescing products associated with "sick" wheat were more readily adsorbed than the compounds responsible for the fluorescence of the endosperm. It had not been possible to establish the utility of any one of the adsorbents to differentiate between the two sources of fluorescence; mainly because the exact amount of fluorescence due to browning could not be determined independently of the fluorescence of naturally fluorescing materials in wheat. The fluorescence due to the "sick" wheat products could only be estimated by subtracting from the total fluorescence reading the reading for an equivalent sample of endosperm of undamaged wheat (or flour).

The question arose as to whether the fluorescence reading for endosperm milled into flour might not differ from that of an equivalent sample of sound whole wheat meal. Furthermore, it had been observed that there is a variation of up to 10 scale units in the fluorescence of different wheat samples (9) and in samples taken from one lot at different moisture levels. These samples were considered representatives of sound wheat.

For these two reasons it was necessary to start out with a sample considered sound. This would furnish a measurement of the background fluorescence. A portion of the starting material would be stored and the rest would be separated into portions showing varying degrees of browning. Fluorescence measurements would be made on all these portions according to the procedure described under METHODS. By subtracting the fluorescence value of the starting material from that of the portions showing varying degrees of browning, the exact amount of fluorescence due to the browning products in each portion would be known. The utility of the adsorbents to differentiate be-

tween the fluorescence due to browning and the background fluorescence could be determined by allowing these to act on the initial sound portion and those having undergone varying degrees of browning.

<u>Effect of Adsorbents on Sound and Browned Germ</u>. The starting material for this procedure was fresh granular wheat germ.

First, a qualitative study was made of several adsorbents on sound and browned germ to observe, in a qualitative manner, which adsorbents would have an effect on either the natural background fluorescence or the fluorescence associated with browning, in the germ. It was interesting to observe that, whereas Isco Adsorbol N-100 and Lloyd Reagent had considerably decreased the fluorescence of the endosperm extracts, their effect on extracts of sound germ was almost nil. Florisil, likewise, appeared to have no effect on extracts of sound germ, while showing very strong adsorption on extracts of browned germ. Unfortunately, the reproducibility of results for this adsorbent was poor. The action of Supercell on extracts of sound and browned germ was exactly as previously observed for extracts of flour and "sick" wheat, i.e., there was no indication of significant adsorption. Norite completely removed the pigments responsible for fluorescence in sound and brown germ extracts.

The results of adsorption studies on a series of four germ samples, processed in an air oven at 50°C for 0, 8, 24, and 48 hours, which had previously been conditioned to a moisture content of 20 percent, are recorded in Table 4.

Data in Table 4 indicate that browning progressed only slightly in the first eight hours, according to fluorescence measurements. It had increased greatly after 24 hours, doubling the initial fluorescence and became about four times as high after 48 hours.

Table 4. Increase in Fluorescence of Germ with Heating Time and Effect of Adsorbents.

Adsorbent	Fluorescence (scale Units)		at various heating time intervals (hours)	
None	16.5	18.0	32.0	64.5
Isco Adsorbol A-420	17.5	17.0	19.0	23.5
Isco Adsorbol N-100	13.0	13.0	15.5	19.0
Lloyd Reagent	16.0	16.5	16.5	19.5

All three adsorbents changed only slightly the fluorescence of the unheated sample, while effecting a strong reduction of the fluorescence of the samples which had undergone heating. Isco Adsorbol N-100 and Lloyd Reagent appeared promising as regards their use in a quantitative determination since (1) the fluorescence of the sound (unheated) fraction had remained relatively unchanged following the use of these adsorbents; (2) the fluorescence of the three fractions subjected to progressive browning was proportionally reduced to a value approaching the fluorescence of the sound (unheated) fraction. Such observations suggested that Isco Adsorbol N-100 and Lloyd Reagent might effect selective removal of the fluorescing browning products, developed with processing time at 50°C; while not adsorbing the compounds responsible for the normal natural background fluorescence of sound wheat germ. Subsequent studies showed that this was not true. Although complete removal of the fluorescing browning products could be effected, such separation was not selective. It became later evident that considerable fluorescence was contributed by these two adsorbents, which varied with the volume and normality of the extraction medium. Since the amount of fluorescence contributed by these adsorbents was

approximately the same as the natural background fluorescence of germ, this led to the erroneous interpretation, at this point, that no adsorption of the compounds responsible for the natural background fluorescence had taken place. That this was not true became evident when the fluorescence of Lloyd Reagent and Isco Adsorbol N-100 was reduced by successive extraction with 0.2 N HCl. When the adsorbents, thus purified, were allowed to act on the extracts of either sound or browned wheat germ, the fluorescence was reduced to zero. This indicated that the fluorescing products associated with browning, as well as those constituting the natural background fluorescence of sound wheat germ, had been indiscriminately adsorbed. No quantitative separation of either of these groups of products was possible.

Use of Florisil Adsorption Columns in the Separation of Fluorescent Products of Wheat Germ. It had been observed, when adsorption studies were begun, that the effect of Florisil on extracts of flour was very slight; whereas strong adsorption followed the action of Florisil on extracts of "sick" wheat or browned germ. Experimentation with this adsorbent had been abandoned because of poor precision. The possibility of increasing the reproducibility of results and of affecting a quantitative separation by the use of adsorption columns was now investigated.

The method of preparing adsorption columns was as follows. A glass column, approximately 11 inches by 0.5 inches was packed with Florisil. Suction was applied at the bottom using a thick walled suction flask, a trap and an aspirator opened to full blast giving maximum suction. The Florisil column was first freed of all interfering fluorescing matter by successive washings with 0.2 N HCl. (0.2 N HCl was also the extraction medium for the wheat germ extracts to be passed through the column. This precluded the

possibility of further release of column impurities due to a change in solvent). Immediately thereafter, while the column was still wet, the extract of sound germ or of germ showing varying degrees of browning, from fluorescence determinations was passed through at 8 ml. increments. It was observed that the first, second, and occasionally, third increments, filtered through the column and caught in the cuvette, were still partially diluted with the solvent previously passed through the column. The results recorded in Table 5 are a summary of fluorescence measurements of the fourth 8 ml. increments passed through the column.

The reduction in the fluorescence following passage through a Florisil adsorption column was measured on four samples representing progressive browning and on one sample which was considered sound.

Data in Table 5 indicate definitely that some separation of fluorescent materials occurs during passage through the column. Although extracts varying over a wide fluorescence range (50 scale units) were poured through the column, the fluorescence of the emerging solutions varied over a very narrow range (1.5 scale units). It appeared that the pigments constituting the natural background fluorescence of wheat germ were eluted with the solvent, whereas the fluorescing products developed as a result of the browning were adsorbed. However, the fluorescence measurement alone can not be considered sufficient evidence of selective adsorption. The fluorescence test, indicative of varying degrees of browning, is restricted to a narrow range. This makes precision all the more important and the results recorded in Table 5, which are averages of several determinations on the same samples, should have agreed more closely since the samples representing progressive browning had been prepared from the same original starting material.

Table 5. Reduction in the Fluorescence of Five Samples Representing Progressive Browning, Following Passage Through a Florisil Adsorption Column.

Treatment	Untreated Sample	Samples representing progressive browning			
None	14.5	19.6	26.3	36.4	64.5
Florisil Adsorption	14.0	13.8	15.3	14.2	15.3

Aside from the limitations of the fluorescence test, there are several other factors which would discourage the use of Florisil in a quantitative evaluation of "sick" wheat deterioration. Some of these factors observed in the course of these studies were that the amount and type of fluorescing materials adsorbed appeared to vary with the rate of flow of solvent. When the solvent was in contact with the column for a longer time, i.e., when suction was interrupted, part of the browning reaction began to appear in the emerging solution, giving high fluorescence readings. Another disadvantage was the extreme slowness of the process.

Florisil had been used to adsorb riboflavin. Rubin and Ritter (28), stated that adsorption was influenced by volume, clarity and concentration of the extracts. Sometimes only partial adsorption occurred, depending on whether riboflavin was in a "free" or combined state. Since there was also a variation between different lots of Florisil, the authors recommended avoiding the use of Florisil in quantitative determinations unless it is clearly established that no losses are incurred for the particular type of sample.

The fact that Florisil adsorbed riboflavin to a more or less extent seemed to limit the chances of obtaining a selective, quantitative separation of the browning reaction products, which could be measured fluorometrically,

aside from other, at present, uncontrollable factors.

Some of these adsorbents, particularly Florisil, may prove valuable in a qualitative analysis and possible identification of some of the compounds formed in the course of the browning reaction in wheat.

Turbidity Method to Follow Changes in Wheat Protein Solubility as Affected by Moisture, Temperature and Processing Time

Since adsorption did not increase the sensitivity of the fluorometric measurement, and the latter apparently does not detect early changes preceding the brown pigmentation in the germ end of the kernel, another independent method was sought to follow the chemical changes in wheat related to the "sick"condition. McDonald had found a decrease in peptizable protein during the browning of wheat germ (McDonald, 22), detectable by changes in turbidity of extracts. Similar decreases in dispersibility by water and dilute salt solutions had been observed as one of the earliest and most marked changes when soybean products were stored above a certain critical temperature (Beckel, et al, 5).

Development of the Method. The turbidity method investigated is based on the Zeleny (33) photometric method for determining the protein content of wheat flour and involves first the extraction of the non-gluten proteins, principally albumins and globulins, in 5 percent potassium sulphate solution. By adding 10 ml. aliquots of the peptized protein to buffers of various pH's, a colloidal suspension (sol) of optimum turbidity and stability was formed at pH 3.4. A buffer of pH 1.7 was used, obtained by mixing approximately 3 parts 0.1 N HCl to 1 part 0.1 N sodium citrate. This buffer, when used in the proportion of 1 ml. buffer to 10 ml. peptized protein, was found to control the pH in the desired region of 3.4, within 0.05 of a pH unit for different

samples and duplicates. The turbidity of suspensions so prepared is determined by reading the percent transmittancy, using the Coleman spectrophotometer. The sample size was arbitrarily fixed at 2.000 g. because it was found that with this sample size the widest possible range of differentiation in percent transmittancy readings could be obtained with samples withdrawn at 24 hour processing intervals.

The detailed procedure developed and used for determining the changes in peptizable protein during processing, was as follows: A 2.000 g. sample of wheat which had been ground to pass through a number 30 screen was shaken intermittently for fifteen minutes with 50 ml. 5 percent potassium sulphate in a 250 ml. glass-stoppered Erlenmeyer flask. The mixture was filtered through a number four Whatman filter paper and 10 ml. of the filtrate were pipetted into a Coleman spectrophotometer tube containing 1 ml. of hydrochloric acid - sodium citrate buffer (pH 1.7). The resulting turbid suspension was allowed to stand for 35 minutes. The percent transmittancy was then read on a Coleman spectrophotometer at a wave length of 530 mu.

Preparation of Samples to Evaluate the Turbidity Method. To evaluate the turbidity method a series of samples were processed to produce varying degrees of deterioration, in the following manner. Five samples of sound wheat of the 1954 Kansas crop were selected. These samples are referred to as C-2, C-5, C-6, C-9 and C-10, (see MATERIALS, p.9) were conditioned to 22 percent moisture content and heated in an air oven at 65°C. The moisture content was determined after tempering. Samples were withdrawn at 24-hour intervals to follow the changes related to the browning reaction in wheat.

The results of moisture determinations on the five samples were the following:

Sample	C-2	C-5	C-6	C-9	C-10
Percent Moisture	21.8	21.5	21.2	21.0	21.9

The changes in peptizable protein with heating time for the five C-series are shown in Table 6. There was only a slight variation in turbidity (percent transmittancy) among the unheated wheats of the five C- series withdrawn just before these samples were subjected to processing at 65°C. The percent transmittancy increased markedly with heating time, indicating almost linear losses in protein solubility with heating time up to 72 hours. After this a leveling off is observed. There appeared to be a slight variation in the rate at which insolubilization occurred for the five C- series. Closer examination of Table 6 reveals that this variation was most apparent between 24 hours and 48 hours of heating time. This difference in the rate of insolubilization may be a reflection of varietal or other differences in these wheat samples.

Table 6. Changes in Peptizable Protein with Heating Time as Measured by the Turbidity Method.

Heating time (hours)	0	24	48	72	96	120
Sample	Percent Transmittancy					
C-2	16.0	44.6	71.8	86.9	90.9	92.0
C-5	13.1	34.2	59.5	86.2	91.6	91.2
C-6	14.9	33.5	64.7	86.5	88.2	90.5
C-9	14.8	37.2	69.5	88.3	91.3	93.0
C-10	14.2	33.0	61.2	86.5	91.9	90.6

A comparison of the rate of insolubilization with the development of fluorescence in the same samples is shown in the data of Fig. 1, which are results for sample series C-2. Figure 1 shows that for every increase in fluorescence there was a corresponding, more extensive change in percent transmittancy.

Since the turbidity method appeared to be a very sensitive index of the early changes during processing at elevated temperatures and moistures, an experiment, similar to the previous one was set up, wherein samples were withdrawn for analyses of fluorescence and turbidity after intervals of 1, 2, 4, 6, 8 and 10 hours processing time. The same conditions of moisture, 22 percent, temperature 65° and variety, C-2, were maintained. Figure 2 illustrates the results obtained. Increases in percent Transmittancy after only one hour denote immediate loss in protein solubility under such conditions. The fluorescence, however, did not change in this time interval. Referring back to Fig. 1, it will be seen that by 24 hours however, the first increase in fluorescence was evident.

Influence of Various Conditions of Treatment of Wheat on Changes in Protein Solubility. An experiment was conducted to determine the first appearance of a decrease in protein peptizability under different conditions of moisture and temperature. For this purpose samples of the C-2 variety were conditioned to moisture levels of 20, 16, and 14 percent were used as well as a sample at the original moisture of 8.3 percent for heating at different temperatures. The samples at four different moisture contents were divided into three fractions and processed in an air oven at 50°C, 65°C, and 100°C. Samples were withdrawn at varying time intervals for turbidity determinations, as recorded in Table 7. The data in Table 7 indicate that there was no decrease in protein solubility in the samples at all moisture

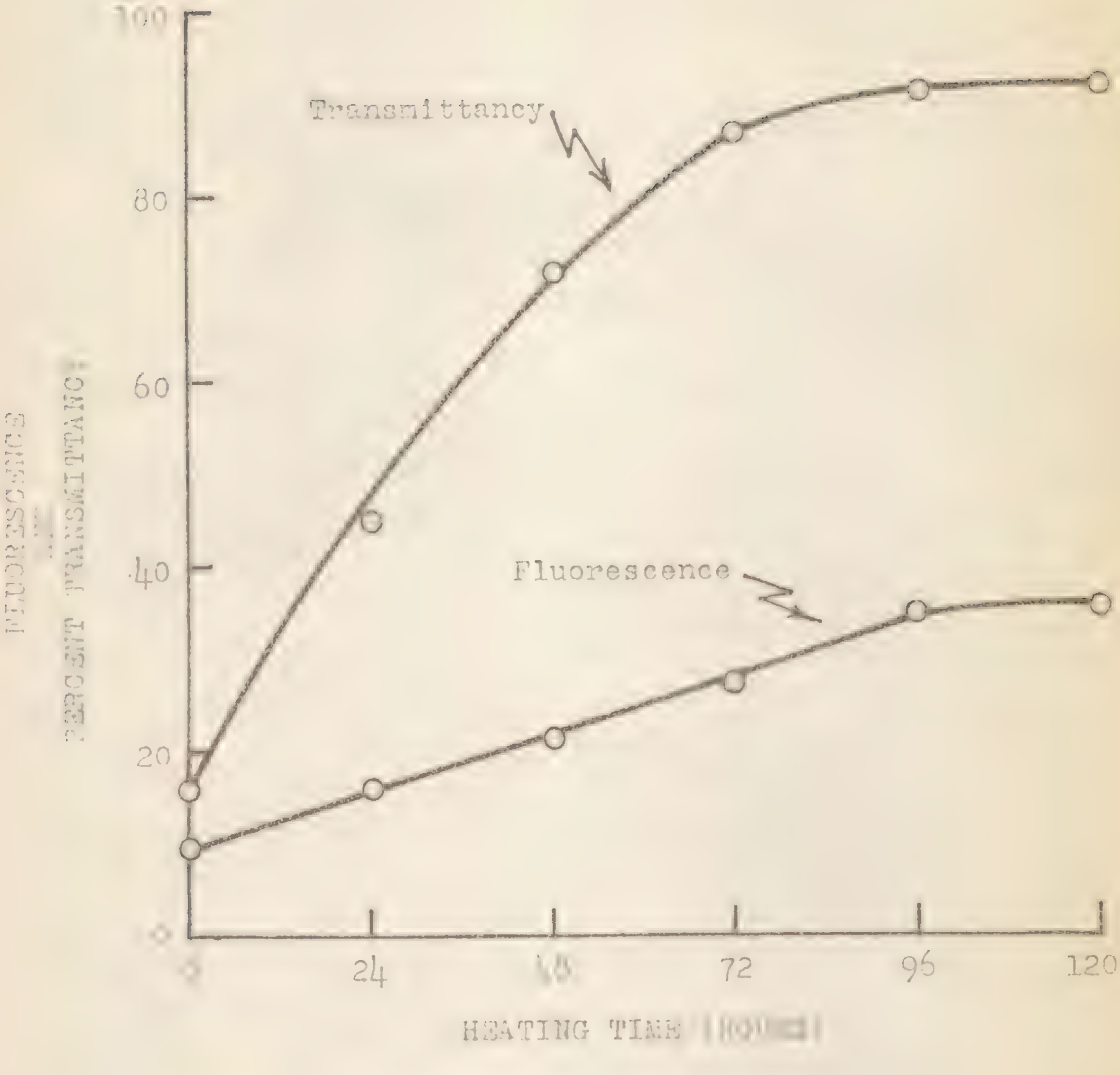
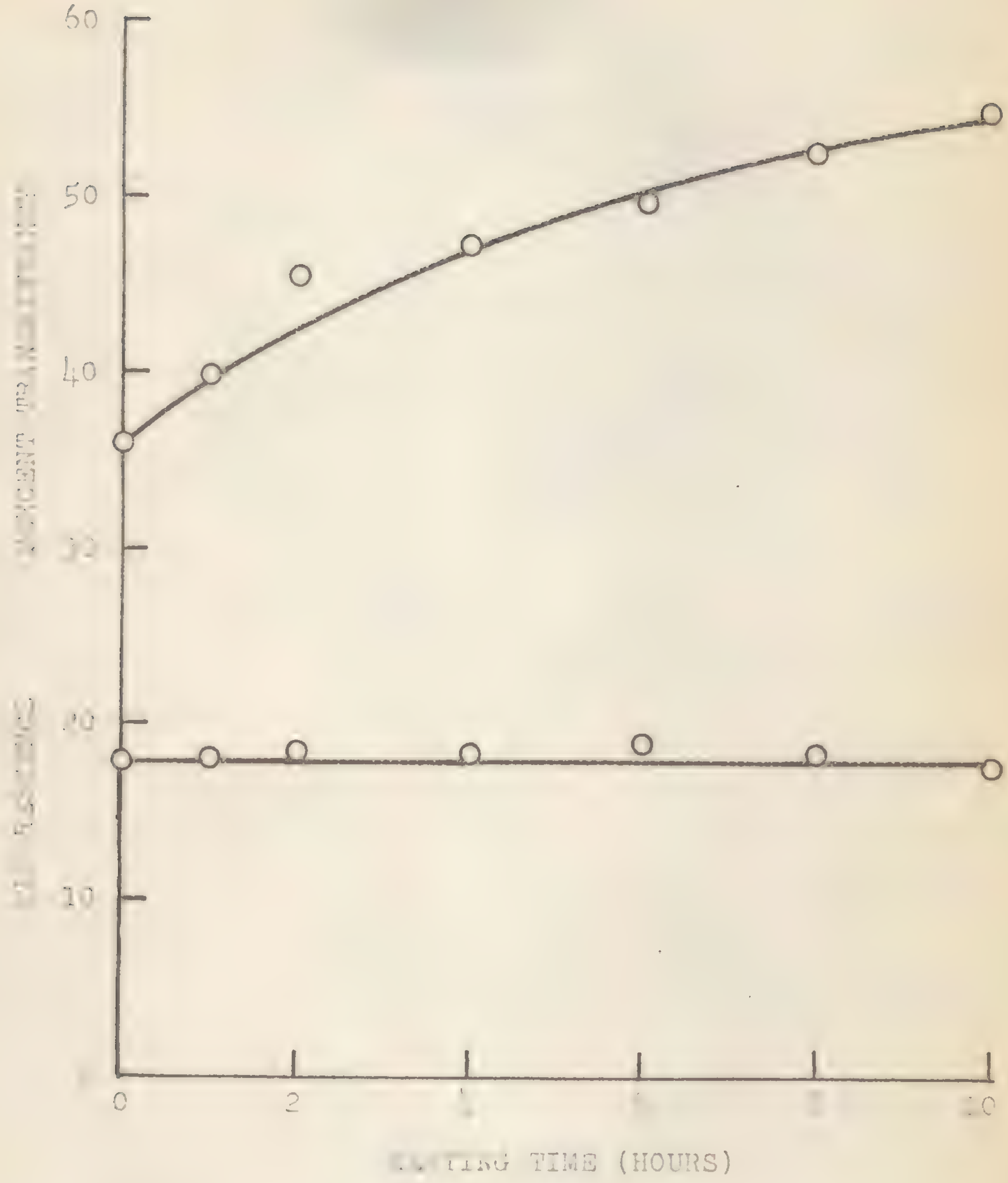


Fig. [illegible] [illegible] in fluorescence and percent transmittancy in extracts of wheat conditioned to a 2[illegible]% moisture content and processed at 6[illegible]°C. (extended treatment)


60
50
40
10
0
2
10
TIME (HOURS)

[illegible]

levels stored at 50° for the duration of the processing time of 24 hours. At 65°, losses in solubility began to be apparent by two hours in samples conditioned to moisture contents of 14 percent and above. These changes became more marked after six hours and 24 hours. At 100°C there was a very marked decrease in protein solubility of the sample containing 20 percent moisture after only one-half hour. Significant decreases had occurred at all moisture levels after two hours at 100°C. An interesting observation was that, whereas no changes at all appeared in the samples processed at 50°C throughout the duration of the experiment (24 hours), the samples processed at 65° began to show a decrease in peptizable protein already after two hours.

This data suggests that there might be a critical temperature above which insolubilization proceeds at a greatly accelerated rate.

Table 7. Protein Insolubilization in Wheat Due to Processing at Various Temperature and Moisture Contents.

Heating time (hours)	Percent Transmittancy at various moisture levels 8.3	14	16	20
		50°C		
2	13.7	13.2	12.8	13.8
6	13.8	15.2	12.0	13.8
24	13.8	13.8	13.5	13.5
		65°C		
2	13.8	17.0	17.5	18.8
6	13.8	20.0	20.2	23.5
24	14.0	25.5	26.9	34.3
		100°C		
0.5	15.5	17.0	17.0	27.5
2	19.2	48.2	50.1	91.5
6	32.0	91.2	91.5	——

DISCUSSION

In considering the use of adsorbents for a more sensitive fluorometric determination of "sick" wheat damage, four or five adsorbents were found which completely removed the browning reaction products. The following were objectives for improving the application of fluorometry to the determination of "sick" wheat damage: (a) Increase the sensitivity to incipient deterioration, which has not been possible previously due to a strong natural background fluorescence of aqueous extracts of wheat and wheat products. (b) Broaden the range of fluorescence between varying degrees progressive browning. These adsorbents which completely removed the browning reaction products were found to also affect the compounds constituting the natural background fluorescence of wheat to a greater or less extent. The browning reaction involves a number of complex processes resulting in a variety of reaction products. For reasons enumerated, it became increasingly apparent in the course of this work that the probability of finding one adsorbent which would quantitatively remove only those products developed as a result of the browning reaction, was very small. Furthermore, it did not seem likely that selective removal of the background fluorescence would broaden the range of fluorescence readings between varying degrees of damage. At best, the detection of incipient stages of deterioration, would be more accurate. It was found that under the particular set of conditions used in this study adsorbents would not serve as an aid in increasing the sensitivity of the fluorometric method. The increase in adsorption was proportional to browning but not selective for the brown substances produced through exposure to elevated temperatures and moistures.

The other possibility, that of removing the natural background fluorescence of wheat and wheat products without affecting the browning products, had long

been abandoned since, invariably, the browning products had been found to become much more firmly adsorbed.

Browned germ and germ-damaged wheat were produced in the laboratory under varying conditions of temperature and moisture. Coincident with the increases in fluorescence--characteristic of progressive browning--there occurred striking decreases in peptizable protein. For every increase in fluorescence, a correspondingly more extensive loss in protein solubility appeared; insolubilization preceded change in fluorescence. To follow the changes in peptizable protein a turbidity method was developed. By this method it was found that the differences in protein solubility among five wheats of different varieties and/or environments were of small magnitude. This is in agreement with the data of Mangels, (20) who determined protein peptized by normal solutions of potassium sulphate, magnesium chloride, potassium bromide and 70 percent alcohol on a series of experimentally milled flours and observed that neither varietal, nor environmental variations in peptizability were of a large magnitude. A variation in the rate of insolubilization was observed, however, when samples of the five wheats were processed under adverse temperature and moisture conditions. A decrease in protein solubility has for many years been regarded as a necessary accompaniment of and a sufficient criterion of denaturation (Neurath, et al 24). The differences observed may be a reflection of varietal or other differences in susceptibility to protein denaturation among these five wheats.

Good evidence has been presented to conclude that heat and moisture alone brought about protein denaturation and the fluorescence increases characteristic of the browning reaction in wheat. The correlation between protein denaturative changes and increases in fluorescence, allow some speculations as to the manner in which heat and moisture induce the browning

reaction in wheat and why this reaction is confined essentially to the germ end of the kernel.

Proteins such as the albumins and globulins are compact, almost globular structures as they occur in nature. The molecules are chainlike but are normally held in a coiled configuration by interaction of numerous intra-molecular bonds. Opportunity for interaction is thus minimal (Senti, et al, 29). Protein denaturation is accompanied by an increased reactivity of constituent groups (24, 25) and by increased susceptibility to enzymatic hydrolysis. It has been suggested that denaturing agents act by rupturing the intramolecular bonds which maintain the folded structure; especially the hydrogen bonds between the carboxyl oxygens and the amide hydrogens of the peptide bonds (Neurath, 19). This results in a fibrillar or fibrous structure with numerous reactive groups. These fibrillar proteins enter into polysaccharide formation much more readily than the globular proteins (27). While denaturation occurs at any temperature, the rate is greatly increased as the temperature rises. In the absence of water the process of denaturation is greatly inhibited. Water is apparently necessary for the mobility of the chains in their structural rearrangement (19).

The brown products, resulting from interaction between amino groups and reducing sugars appear primarily in the germ rather than the endosperm probably for the following reasons.

(1) No significant amounts of simple, reducing sugars are normally present in the endosperm or the bran portion. Such sugars would only appear due to amylase action or other type of starch degradation. Therefore, even if protein denaturation should proceed in the endosperm or the bran, browning would not occur due to the unavailability of reactive sugars in those tissues.

(2) The germ contains approximately 25 percent of simple sugars, sucrose (which could yield reducing sugars by hydrolysis) and raffinose (Bailey, 4).

(3) The heat-coagulable proteins, globulin and the albumin leucosin, constitute the greater part of the germ protein (26). Globulins and albumins are practically non-existant in the endosperm.

The effect of heat and moisture in inducing the browning reaction in wheat may involve one or several of the following steps:

The denatured proteins react readily with sugars in the germ;

Hydrolysis of sucrose, yielding increased amounts of reducing sugars;

Modifications in the sugars caused by the presence of "free" amino groups;

The observation of some variation in the rate of protein insolubilization among five different wheat samples suggests that different wheats, although all perfectly sound, may show variable susceptibility to germ-damage when stored under identical conditions. Further study of this possibility is necessary.

SUMMARY

A study was carried out of the utility of adsorbents to separate the fluorescence which appears in the wheat embryo with the deterioration commonly known as "sick" wheat from the natural fluorescence characteristic of the balance of the kernel. Investigation was also made of a turbidity method to detect early changes in wheat related to the "sick" wheat condition. The following results were obtained:

(1) Of about 19 adsorbents investigated, four or five completely removed the products associated with "sick" wheat deterioration, as measured by fluorometry.

(2) None of these adsorbents were selective for the browning reaction products, since the substances constituting the background fluorescence were also affected to some extent.

(3) Browned germ and germ-damaged wheat could be produced in the laboratory by processing at elevated moisture contents and temperatures for various lengths of time.

(4) Progressive browning was associated with extensive increases in percent transmittancy due to protein insolubilization.

(5) Losses in peptizable protein preceded measurable increases in fluorescence.

(6) Differences in peptizable protein among five untreated sound wheat samples were very slight. There appeared to be a variation in the rate of insolubilization when the samples were heat-treated at elevated moisture content.

(7) Decreases in peptizable protein were observed in as little as two hours, when intact kernels conditioned to various moisture levels were heated at $65^{\circ}C$. No changes were observable even after 24 hours when intact kernels of the same variety and at identical moisture levels, were heated at $50^{\circ}C$.

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A STUDY OF CHANGES IN FLUORESCENCE AND PROTEIN SOLUBILITY IN GERM-DAMAGED WHEAT

by

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A type of damage occurring in wheat stored at high levels of moisture and temperature has been known in the trade as "sick" wheat. The affected kernels take on a dull appearance and the germs exhibit various degrees of discoloration from light brown to black. The formation of "sick" wheat is associated with the production in germ of wheat of substances which are strongly fluorescent, apparently produced by a Maillard or browning reaction. Based on the increased fluorescence of aqueous extracts of "sick" wheat to values above that for sound wheat, a fluorometric technique had been developed to furnish an objective evaluation of the degree of "sick" wheat damage. Since there are also naturally fluorescing substances in wheat, which interfere with the measurement of the fluorescence increase associated with germ damage, the fluorometric technique was not sufficiently sensitive to detect deterioration in its critical initial stages.

The purpose of the present research was to develop a more sensitive method of detecting the degree of damage in a sample of "sick" wheat, particularly in its initial stages.

An investigation of the use of adsorbents to separate the products of deterioration associated with the browning of wheat germ from naturally fluorescing substances in wheat, and thus increase the sensitivity of the fluorometric technique, resulted in the finding of four to five adsorbents which completely removed the browning reaction products. However, adsorption was not selective; the natural constituents of wheat were also affected to a more or less extent.

Browned germ and germ-damaged wheat were produced in the laboratory by conditioning fresh granular germ and intact wheat kernels to elevated moisture contents (up to 22 percent) and heating the grain at temperatures from 50°C

to 100°C for varying lengths of time. Several series representing progressive browning under controlled conditions were thus obtained.

Progressive browning was characterized by marked changes in peptizable protein in addition to the increases in fluorescence.

A method was developed to follow these changes, based on the decreasing dispersibility by dilute salt solutions (5 percent potassium sulphate solution) of proteins of wheat subjected to elevated temperatures. The change in protein dispersibility was determined by measurement of the turbidity of extracts brought to pH 3.4, as indicated by reading the percent transmittancy at a wave length of 530 mu.

The losses in peptizable protein, indicated by increases in percent transmittancy due to protein insolubilization, preceded measurable increases in fluorescence and were in all instances more extensive than the increases in fluorescence.

The measurement of protein insolubilization was found to be a most sensitive index for following the initial changes associated with germ deterioration in wheat which was browned in the laboratory. This method may become a valuable aid for detecting incipient deterioration in commercial "sick" wheat or in predicting susceptibility to germ deterioration.